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MPMI Vol. 24, No. 8, 2011, pp. 867–878. doi:10.1094/MPMI-01-11-0019.

CURRENT REVIEW

Lipo-chitooligosaccharide Signaling in Endosymbiotic Plant-Microbe Interactions

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Submitted 28 January 2011. Accepted 19 March 2011.

The arbuscular mycorrhizal (AM) and the rhizobia-legume (RL) root endosymbioses are established as a result of signal exchange in which there is mutual recognition of diffusible signals produced by plant and microbial partners. It was discovered 20 years ago that the key symbiotic signals produced by rhizobial bacteria are lipo-chitooligosaccharides (LCO), called Nod factors. These LCO are perceived via lysin-motif (LysM) receptors and activate a signaling pathway called the common symbiotic pathway (CSP), which controls both the RL and the AM symbioses. Recent work has established that an AM fungus, *Glomus intraradices*, also produces LCO that activate the CSP, leading to induction of gene expression and root branching in *Medicago truncatula*. These Myc-LCO also stimulate mycorrhization in diverse plants. In addition, work on the nonlegume *Parasponia andersonii* has shown that a LysM receptor is required for both successful mycorrhization and nodulation. Together these studies show that structurally related signals and the LysM receptor family are key components of both nodulation and mycorrhization. LysM receptors are also involved in the perception of chitooligosaccharides (CO), which are derived from fungal cell walls and elicit defense responses and resistance to pathogens in diverse plants. The discovery of Myc-LCO and a LysM receptor required for the AM symbiosis, therefore, not only raises questions of how legume plants discriminate fungal and bacterial endosymbionts but also, more generally, of how plants discriminate endosymbionts from pathogenic microorganisms using structurally related LCO and CO signals and of how these perception mechanisms have evolved.

Plants form root symbioses with soil microorganisms, leading to improved plant nutrition and increased productivity in nutrient-limiting conditions. Root symbioses are classified into either endosymbioses, in which the microbes penetrate plant cells to establish an intracellular symbiosis, or exo- or ectosymbioses, in which they remain outside. During the last 20 years, remarkable progress has been made in the understanding of the molecular and cellular events that lead to the development of two major plant root endosymbioses, the arbuscular mycorrhizal (AM) and the rhizobia-legume (RL) symbioses (Bonfante and Genre 2010; Ferguson et al. 2010; Oldroyd and Downie 2008; Parniske 2008).

The AM symbiosis is formed between fungi of the Glomeromycota phylum (such as *Glomus* and *Gigaspora* species) and a wide range of plants, including angiosperms, gymnosperms, pteridophytes, and some bryophytes and liverworts (Harrison 2005; Humphreys et al. 2010). This symbiosis is obligatory for the microsymbiont, which cannot develop and fulfill its life cycle in the absence of a host plant. Establishment of the AM symbiosis involves the formation of a fungal hyphopodium on the root surface, followed by the initiation of a polarized cytoplasmic assembly in underlying plant cells called the prepenetration apparatus (PPA), through which fungal hyphae penetrate plant cells (Genre et al. 2005). In the root inner cortical cells, the hyphae branch dichotomously to form highly branched structures called arbuscules, in which nutrient exchange takes place. The AM symbiosis allows the plant to benefit from the external fungal mycelium to scavenge for nutrients, particularly phosphorus, nitrogen, and sulphur (Harrison 2005). The RL symbiosis is essentially restricted to members of the Fabaceae (legume family) and a variety of phylogenetically diverse gram-negative bacteria, termed rhizobia (Markmann and Parniske 2009; Masson-Boivin et al. 2009; Sprent 2007). However some tropical trees in the nonlegume genus *Parasponia*, intriguingly, also form this symbiosis. The RL symbiosis leads to the development of root nodules in which the bacteria fix dinitrogen to the benefit of host plants. Nodule organogenesis and infection are separable processes that are highly coordinated (Oldroyd and Downie 2008). Rhizobial infection shows considerable variation between plant species (Sprent 2007) but, in many cases, involves the formation of plant-derived infection threads that carry the bacteria into the dividing cells of the nodule primordium (Oldroyd and Downie 2008). For the initial step of infection at the root epidermis in some plants, the rhizobia enter via "crack entry" at sites of lateral and adventitious root emergence (Goormachtig et al. 2004), but many plants show a more advanced mode of entry via the initiation of infection threads in root hairs that have curled around the bacteria (Oldroyd and Downie 2008). The cell-to-cell progression of infection threads is then determined by preinfection threads, which bear cytological similarities to the PPA. Other plant species (known as actinorhizal plants), from several families of the Fabidae clade of higher plants, form a nitrogen-fixing symbiosis with gram-positive bacteria of the genus *Frankia*. Like the RL symbiosis, the actinorhizal symbiosis involves nodulation and epidermal infection, either via root hairs or by crack entry, depending on the host plant (Kucho et al. 2010; Markmann and Parniske 2009).

The facultative nature of the RL symbiosis and the amenable genetics of the bacterial and legume symbionts led to the identification of a molecular dialog that controls mutual recognition of the partners followed by development of nodulation. Host root–secreted molecules (generally flavonoids) are recog-

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nized by rhizobia, leading to the synthesis and secretion of lipo-chitooligosaccharide (LCO) symbiotic signals, called Nod factors (D'Haeze and Holsters 2002; Dénarié et al. 1996; Lerouge et al. 1990). Specific recognition by host roots of rhizobial Nod factors, in turn, sets into motion the infection process and nodule organogenesis (Oldroyd and Downie 2008). Nod factors bear similarities in structure to chitooligosaccharide (CO) elicitors, which are derived from fungal cell walls and activate defense responses in many plants. CO are an example of a microbial-associated molecular pattern, which leads to pattern-triggered immunity and defense responses to invading pathogens (Boller and Felix 2009; Hamel and Beaudoin 2010). The structural similarity between Nod factors and CO raises the question of how specificity is achieved in the activation of symbiotic and defense pathways.

Studies, particularly in the model legumes *Medicago truncatula* and *Lotus japonicus*, led to the identification of candidate Nod factor receptor genes and genes encoding components of Nod factor signaling (Kouchi et al. 2010). Some of the signaling genes also control development of the AM symbiosis, thus defining the so-called common symbiotic pathway (CSP) and showing that activation of this pathway is a prerequisite for establishing these plant-root endosymbioses. In the AM symbiosis, root-secreted strigolactones induce branching and increased metabolism of fungal hyphae (Akiyama et al. 2005; Besserer et al. 2006; Xie et al. 2010). By analogy to Nod factor signaling, it was hypothesized that AM fungi would secrete symbiotic signals, termed Myc factors, which would activate the CSP (Catoira et al. 2000). Indeed, several reports have shown that AM fungi secrete diffusible molecules that induce various responses in plant roots, some of which are CSP-dependent (Chabaud et al. 2011; Gutjahr et al. 2009; Kosuta et al. 2008; Kuhn et al. 2010; Oláh et al. 2005; Weidmann et al. 2004).

Here, we review the first structural identification from *Glomus intraradices* of mycorrhizal signals that activate the CSP pathway in *M. truncatula* (Maillet et al. 2011). We compare the mechanisms of signaling by Nod factors and these Myc factors and review the recent work on perception of LCO

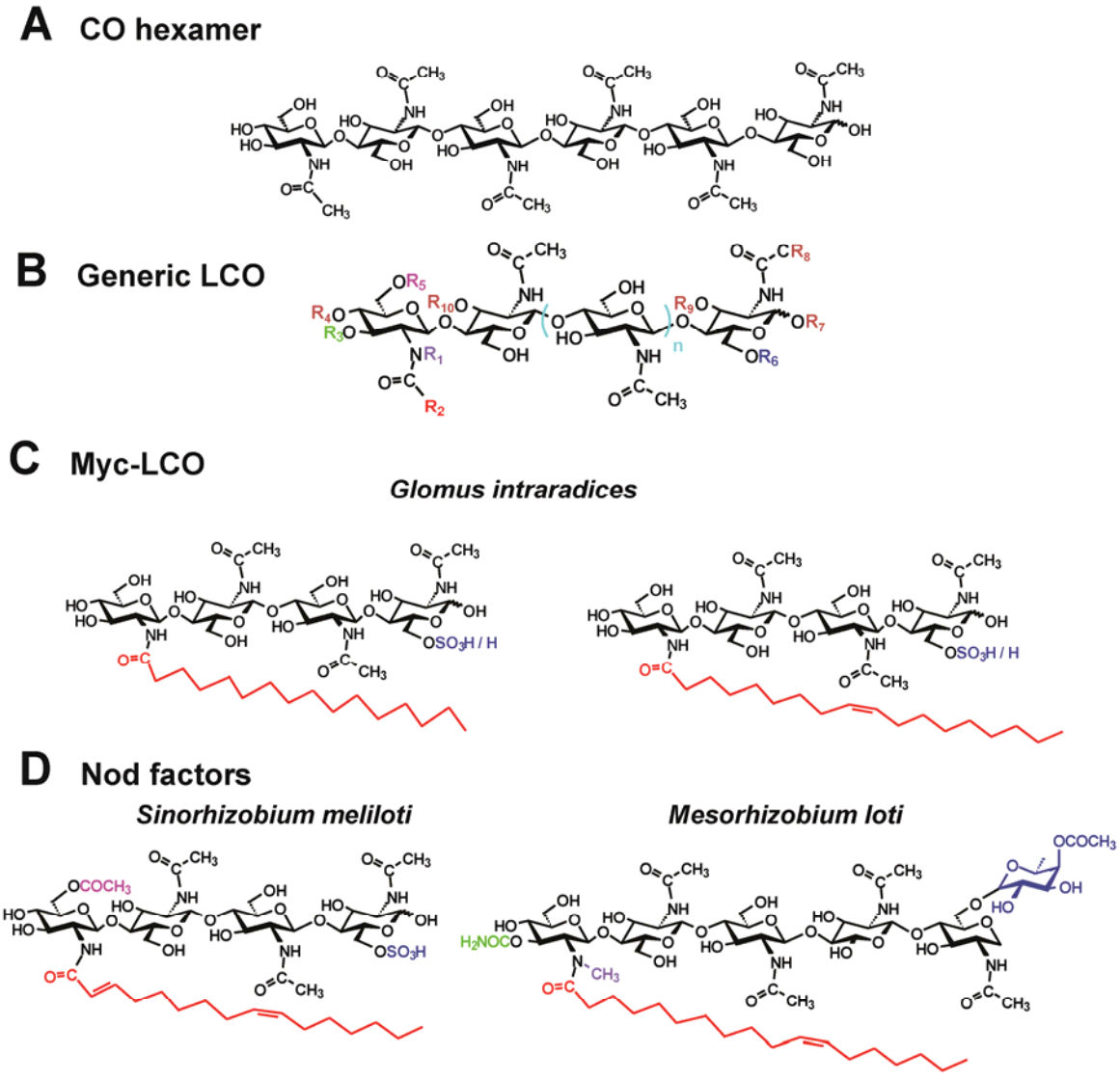

Fig. 1. Structure of chitooligosaccharides (CO) and lipo-chitooligosaccharides (LCO). **A,** Structure of a CO hexamer, showing alternating orientation of the GlcNAc residues and linearity of the GlcNAc oligomer due to the β1-4 linkages. **B,** A generic symbiotic LCO showing sites of chemical substitutions. *n* is generally 1 or 2, leading to substituted LCO tetramers or pentamers. **C,** The structure of sulphated and nonsulphated Myc-LCO of *Glomus intradices*: Myc-LCO-IV (C16:0, +/–S) and Myc-LCO-IV (C18:1Δ9Z, +/–S) (Maillet et al. 2011). Note that similar pentameric molecules have also been identified. **D,** The structure of major Nod factors of *Sinorhizobium meliloti* NodSm-IV(C16:2Δ2EΔ9Z, Ac, S) (Lerouge et al. 1990; Truchet et al. 1991) and *Mesorhizobium loti* NodMl-V (C18:1Δ11Z, Me, Cb, AcFuc) (Bek et al. 2010; Lopez-Lara et al. 1995).

and CO and how structurally related signals can lead to cross-talk and specific responses. We also discuss the evolution of the AM and RL endosymbioses from recent studies on symbiotic signals and receptors.

Symbiotic LCO signals: structural variation.

Based on the hypothesis that AM fungi produce symbiotic signals structurally related to Nod factors, Maillet and associates (2011) used bioassays specific for sulphated and nonsulphated LCO to detect such compounds in extracts of *Glomus intraradices*. These assays were used to purify active fractions that were subjected to mass spectrometry identification of the LCO. Germinated spore extracts of *G. intraradices* yielded small amounts of compounds characteristic of chitin tetramers substituted with either oleic acid (LCO-IV C18:1Δ9Z) or palmitic acid (LCO-IV C16:0) on the terminal nonreducing sugar, and evidence was obtained for the presence of sulphated LCO (Fig. 1). Exudates of carrot root cultures colonized with *G. intraradices* also yielded sulphated compounds that corresponded to a mixture of tetramers and pentamers (about 10-fold more tetramers than pentamers) with C16 or C18 chains with zero, one, or two unsaturations. In addition, the same tetrameric nonsulphated LCO were observed as in the spore extracts. No such LCO were detected in extracts of noncolonized roots. Together these results show that an AM fungus secretes a mixture of sulphated and nonsulphated tetrameric and pentameric LCO, with acyl chains that correspond to quite common fatty acids in fungi (Olsson and Johansen 2000).

In order to facilitate the study of the biological activity of Myc-LCO, chemical mimetics were produced, either by the *Escherichia coli*–cell factory production of modified CO followed by chemical acylation (Ohsten Rasmussen et al. 2004) or by using specific rhizobial mutant strains. These techniques have been used previously to produce different Nod factor mimetics for structural and biological activity studies. The sulphated and nonsulphated Myc-LCO mimetics produced by the cell-factory approach were chemically identical to the major Myc-LCO shown in Figure 1 and contained about 10% pentamers. The Myc-LCO mimetics were shown to increase mycorrhization in three plant hosts, *Medicago truncatula* (barrel medic, family Fabaceae), *Tagetes patula* (French marigold, family Asteraceae), and root cultures of *Daucus carota* (carrot, family Umbelliferae), thus suggesting that the *G. intraradices* LCO are mycorrhizal signals that are active in a broad range of plants.

These Myc-LCO are structurally remarkably similar to Nod factors as both signals have the same basic LCO structure of a chitin tetramer or pentamer (linear arrays of β1-4-linked *N*-acetyl glucosamine [GlcNAc] residues) in which the *N*-acetyl group on the terminal nonreducing sugar is replaced with an N-linked C16 or C18 acyl chain (Fig. 1). For Nod factors, each rhizobia produces LCO structures with variations that are characteristic of a rhizobial strain or species (D'Haeze and Holsters 2002; Dénarié et al. 1996). Variations occur in the chemical substitutions on the oligosaccharide moiety (*O*-linked acetyl, carbamoyl, fucosyl, arabinosyl, or sulphuryl groups and *N*-linked methyl groups) and in the length and structure of the acyl chain (often *cis*-vaccenic acid [C18:1Δ11Z] or chains with trans double bonds conjugated to the carbonyl group). The specific substitutions are important for host range and for biological activities. The major Nod factors produced by *Sinorhizobium meliloti* [NodSm-IV(C16:2Δ2EΔ9Z, Ac, S)] and *Mesorhizobium loti* [NodMl-V(C18:1Δ11Z, Me, Cb, AcFuc)], the symbionts of *M. truncatula* and *L. japonicus*, respectively, are shown in Figure 1. For *S. meliloti*, sulphation of the Nod factors is essential for nodulating *Medicago* plants and it should be noted that all symbiotic Nod factor activities on these plants show a high degree of specificity for sulphated factors. In addition, the structure of the acyl chain and the presence of the *O*-acetyl group are important for infection (Ardourel et al. 1994). No rhizobia strain has been shown to produce Nod factors with an oleic acid (C18:1Δ9Z) chain, thus providing what could be an important difference between factors of rhizobial or fungal origin (Maillet et al. 2011). Interestingly, rhizobia with broad host-ranges generally produce both sulphated and nonsulphated Nod factors, suggesting that the production of sulphated and nonsulphated Myc-LCO by *G. intraradices* may be important for the wide host range of the fungus.

Nod and Myc-LCO are structurally related to CO elicitors (Fig. 1), which have been shown to induce a variety of defense responses in many plant species. However, whereas the symbiotic factors are generally based on four and five GlcNAc residues, the most active chitin elicitors have larger degrees of polymerization. For example, studies in rice and *Arabidopsis* have shown that six- to eight-residue CO are more active in eliciting gene expression and other responses than ones with three to five GlcNAc residues (Shibuya and Minami 2001; Zhang et al. 2002). There are also considerable differences in the concentrations of these molecules that elicit responses. Nod factors are active in some bioassays at concentrations down to 10^{-13} M, Myc-LCO down to 10^{-11} M, whereas many studies on CO responses have used concentrations of between 10^{-9} to10^{-6} M.

Structural studies of LCO by nuclear magnetic resonance and molecular dynamics simulations have shown that the saccharidic moiety is essentially linear, but with an important amount of conformational freedom around the β1-4 glycosidic linkages (Gonzalez et al. 1999; Morando et al. 2011). Addition of an acyl chain makes the molecule amphipathic and allows it to form aggregates at high concentrations (10^{-3} M) (Groves et al. 2005), thus suggesting that the Nod factors are likely to be monomeric at physiological concentrations. Nod factors also spontaneously insert into artificial membranes (Goedhart et al. 1999), but as the structural variation in the acyl chain is important for Nod factor activities, it is likely that the acyl chain is involved in the specificity of perception rather than membrane insertion. Indeed, in vitro and in silico studies have shown that

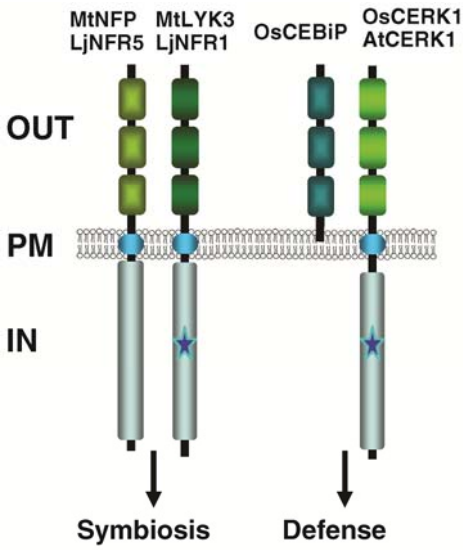

Fig. 2. Schematic representation of the predicted domain structures of lipo-chitooligosaccharide (LCO) and chitooligosaccharide (CO) receptor proteins. Proteins are shown inserted into the plasma-membrane (PM); the three lysin-motif (LysM) domains are extracellular (OUT) and the kinase domains intracellular (IN). Stars in the intracellular domains represent active kinase domains. AtCERK1 is also known as AtLysM RLK1.

the structure of the acyl moiety can affect the shape of the LCO molecule such that the relative orientation of the oligosaccharidic and acyl moities can range from being extended to essentially perpendicular to being quasi-parallel (Groves et al. 2005; Morando et al. 2011). Thus small differences in the acyl chains could be very important for the specificity of the perception and biological activities of different Nod factors and Myc-LCO.

LCO and CO receptors: the same family of proteins.

Forward genetics on model legumes and on *Arabidopsis* have identified lysin motif (LysM) proteins as key components of Nod factor and CO perception. LysM domains occur in a variety of proteins in bacteria and eukaryota and have been shown to bind GlcNAc containing ligands (Buist et al. 2008; Ohnuma et al. 2008). Only in plants are LysM domains associated with a kinase-like domain to form two main LysM-RLK gene families which have been termed the LYK (LysM-I or LYS-I) and the LYR (LysM-II, Lys-II) families (Arrighi et al. 2006; Lohmann et al. 2010; Shiu et al. 2004; Zhang et al. 2007). In addition, plants contain other LysM proteins that do not possess a kinase-like domain (Zhang et al. 2007). All the LysM-RLK are predicted to contain three LysM domains, although these domains exhibit a high degree of divergence, both within a protein and between proteins (Arrighi et al. 2006; Lohmann et al. 2010; Zhang et al. 2007). In *M. truncatula* and *L. japonicus*, both a *LYR* gene (*MtNFP* or *LjNFR5)* and a *LYK* gene *(MtLYK3* or *LjNFR1)* are required for Nod factor–dependent nodulation and infection (Arrighi et al. 2006; Limpens et al. 2003; Madsen et al. 2003; Radutoiu et al. 2003) (Fig. 2). Closely related genes are involved in Nod factor perception in pea and soybean (Indrasumunar et al. 2010; 2011; Madsen et al. 2003; Zhukov et al. 2008).

Recently, work on the nonlegume *Parasponia andersonii* has shown that a *LYR* gene, *PaNFP*, which is closely related to *MtNFP* and *LjNFR5,* is required for both nodulation and mycorrhization (Op den Camp et al. 2010). In plants in which the *PaNFP* RNA is knocked down by RNAi, rhizobia are still able to penetrate roots intercellularly, but an intracellular symbiosis is not established. Similar experiments showed that hyphae of *Glomus intraradices* penetrated the roots of such transgenic RNAi plants but the hyphae did not develop to form arbuscules. Although it is difficult from these RNAi experiments to assess the role of PaNFP in initial symbiotic events, the results show that the same LysM-RLK gene is involved in intracellular infection for establishment of both the AM and the RL symbioses (Op den Camp et al. 2010).

Consistent with the role of a *LYR* gene in mycorrhization, the recent work on Myc-LCO with *M. truncatula* suggests that *MtNFP* is partially involved in perceiving Myc-LCO, leading to stimulation of root branching (Maillet et al. 2011). Sulphated Myc-LCO are about 100-fold more active than nonsulphated ones in this response and, at 10^{-9} M sulphated and 10^{-8} M nonsulphated Myc-LCO concentrations, the response is abolished in *nfp* mutants. At higher concentrations (10^{-7} M) of nonsulphated Myc-LCO, *MtNFP* is partially dispensable for the root-branching response, suggesting that, in these conditions, a higher abundance but lower affinity receptor would operate. This is consistent with the fact that *MtNFP* is not essential for mycorrhization (Ben Amor et al. 2003) and suggests that another LysM protein may act as a Myc factor receptor. In this context, it is noteworthy that biochemical studies have identified two different LCO binding sites in *M. truncatula* roots (Bono et al. 1995; Hogg et al. 2006) and another one in the plasma-membrane of *Medicago* cell cultures (Gressent et al. 1999) that recognize both sulphated and nonsulphated Nod factors and which are independent of *MtNFP* (Hogg et al. 2006). Such sites could be involved in Myc-LCO perception (Hogg et al. 2006). In addition, MtNFP-independent signaling is implicated in the stimulation of some plant responses by AM fungal spore exudates (Chabaud et al. 2011; Mukherjee and Ané 2011; Olah et al. 2005). This signaling (which is CSP-dependent [discussed below]) could be explained by redundancy between MtNFP and another receptor. Interestingly, the *M. truncatula* LysM-RLK gene *LYR1* is implicated in mycorrhization by expression data (Gomez et al. 2009). Alternatively, AM fungi could produce other symbiotic signals that are not perceived by MtNFP.

To date no direct binding of Nod factors has been shown to the putative Nod factor receptors, but genetic experiments strongly implicate the LysM domains of the symbiotic LysM-RLK in discriminating different Nod factor structures characteristic of their corresponding symbionts. In *L. japonicus*, both LjNFR5 and LjNFR1 are required for initial Nod factor responses, and transfer of these two genes to *M. truncatula* enables these transgenic plants to be nodulated by the *Lotus* symbiont, *Mesorhizobium loti* (Radutoiu et al. 2007). Further analysis of nodulation of different *Lotus* species, identified the LysM2 domain of LjNFR5 as critical for perception of Nod factor structures (Bek et al. 2010; Radutoiu et al. 2007). In *M. truncatula*, only MtNFP is required for initial signaling responses, whereas both MtNFP and MtLYK3 are required for infection, which has a higher stringency for the Nod factor structure (Ardourel et al. 1994; Arrighi et al. 2006; Limpens et al. 2003; Smit et al. 2007). Recent work suggests that the precise structure of LysM2 of MtNFP is particularly important for infection but not for the initial signaling response (S. Bensmihen, *personal communication*). In addition, the role of MtLYK3 in infection involves the recognition of chemical substitutions on the terminal nonreducing sugar (acyl chain structure and C6 *O*-acetylation) of Nod factors (Limpens et al. 2003; Smit et al. 2007). Related *LYK3*-like genes in pea are implicated in the discrimination of factors containing an *O*-acetyl group on C6 of the reducing sugar (Limpens et al. 2003; Smit et al. 2007; Zhukov et al. 2008). Thus, both members of the pairs are implicated in Nod factor perception, although it cannot be excluded that additional proteins may also be involved, particularly in the specificity of responses. For example, a Nod factor binding apyrase protein has been described in *Dolichos biflorus* and this and a related protein in soybean seem to have early symbiotic roles (Etzler et al. 1999; Govindarajula et al. 2009). Recently, an E3 ubiquitin ligase of the U-box family, PUB1, has been shown to interact with MtLYK3 and increases the specificity of nodulation in *M. truncatula* to rhizobial symbionts producing wild-type Nod factors (Mbengue et al. 2010).

In *Arabidopsis* a LysM-RLK termed CERK1 (Miya et al. 2007) or LysM RLK1 (Wan et al. 2008) is required for CO responses and for resistance to fungal pathogens (Petutschnig et al. 2010) (Fig. 2). In rice, the major chitin elicitor–binding protein in biochemical studies is a related LysM protein (CEBiP) that does not contain an intracellular region but appears to be attached to the external side of the plasma-membrane (Kaku et al. 2006) (Fig. 2). The rice CEBiP protein is essential for CO responses (Kaku et al. 2006), contributes to disease resistance to a fungal pathogen (Kishimoto et al. 2010), and forms heterooligomers with OsCERK1 after chitin elicitor treatment (Shimizu et al. 2010). Since no CO binding has been shown to OsCERK1, chitin elicitors could be primarily perceived in rice by CEBiP, followed by signal transduction via the active kinase domain of OsCERK1 (Shimizu et al. 2010) (Fig. 2). In *Arabidopsis*, AtCERK1 binds insoluble chitin and also weakly binds CO (Iizasa et al. 2010; Petutschnig et al. 2010). Whether responses at low CO concentrations

require a CEBiP-like protein in *Arabidopsis* is not yet clear, as such a protein has not been detected biochemically (Miya et al. 2007), even though there are three predicted in the genome (Wan et al. 2008).

From the two Nod factor-receptor gene pairs, sequence and biochemical analysis suggest that only the MtLYK3 and LjNFR1 proteins have an active kinase domain, which is essential for its function in autophosphorylation and activation of downstream responses (Arrighi et al. 2006; Klaus-Heisen et al. 2011; Madsen et al. 2011). MtNFP and LjNFR5 have dysfunctional kinases and may thus initiate downstream responses by modifying the activity of a protein interactor through conformational changes rather than protein phosphorylation activity. Recently, LjNFR5 and LjNFR1 have been shown to form heterooligomers, and the kinase domain of LjNFR1 phosphorylated the intracellular region of LjNFR5 in vitro (Madsen et al. 2011). Thus, LjNFR5 and LjNFR1 as well as MtNFP and MtLYK3 may form signal transduction pairs as for OsCEBiP and OsCERK1 (Fig. 2). In *M. truncatula*, an additional LYK partner for MtNFP would need to be invoked for early Nod factor signaling, as MtLYK3 is not required for these initial responses (Catoira et al. 2001; Smit et al. 2007) (Fig. 3). For mycorrhization, the *Parasponia PaNFP* gene lacks certain features characteristic of active kinases (Op den Camp et al. 2010) and therefore, like MtNFP and LjNFR5, it, too, is likely to possess an inactive kinase domain, thus raising the question of how the Myc signal recognized by PaNFP is transduced to activate downstream responses.

Symbiotic LCO signaling: common and specific pathways.

The common symbiotic pathway. The CSP that controls both nodulation and mycorrhization is activated by Nod factors via LysM-RLK proteins. Eight proteins in *L. japonicus* and four in *M. truncatula* are, so far, known to constitute the CSP (Kouchi et al. 2010). CSP components are also conserved and essential for mycorrhization in rice (Banba et al. 2008; Chen et al. 2007, 2008, 2009; Gutjahr et al. 2008), and one CSP component is known so far in actinorhizal plants that are nodulated by *Frankia* species (Gherbi et al. 2008; Markmann et al. 2008). CSP proteins can be classified into two groups, one positioned upstream and the other downstream of calcium spiking, which is periodic oscillations of calcium both in and around the nucleus (Oldroyd and Downie 2006; Sieberer et al. 2009). The upstream proteins required for calcium spiking are a leucine-rich repeat RLK (MtDMI2 and LjSYMRK), components of a nucleoporin complex (LjNUP85, LjNUP133, and LjNENA), and nuclear-membrane localized ion channels (MtDMI1 and LjCASTOR, LjPOLLUX). Downstream of calcium spiking, there is a nuclear calcium and calmodulin-dependent protein kinase (CCaMK) (MtDMI3/LjCCaMK) and a nuclear coiled-coil protein (MtIPD3/LjCYCLOPS) that interacts with the CCaMK to decode calcium spiking for downstream signaling. Downstream of the CSP, transcription factors of the GRAS family, namely, NSP1 and NSP2, have been reported to be specific for nodulation in *M. truncatula* and *L. japonicus* (Heckmann et al. 2006; Kalo et al. 2005; Smit et al. 2005) (Fig. 3).

Several studies have shown that diffusible AM fungal factors induce plant responses. Some of these are CSP-independent, notably in rice (Gutjahr et al. 2008; Mukherjee and Ané 2010), but also in *M. truncatula* (Kosuta et al. 2003; Kuhn et al. 2010). The potential symbiotic significance of such signals is currently not known, although this could be assessed by testing whether they stimulate mycorrhization and whether the characterized responses are still induced in other mycorrhization-deficient plant mutants that should become available in the future. In contrast, the requirement of the CSP in establishing the AM symbiosis in both dicots and rice testifies to the importance of signals that stimulate responses via the CSP. Indeed, the Myc factor hypothesis was reinforced by the finding that, just like Nod factors, diffusible AM fungal factors can induce plant responses in a CSP-dependent manner. For example, the stimulation of root branching in *M. truncatula* by nonpurified diffusible AM fungal factors was shown to depend on two CSP genes and not on *MtNSP1*, indicating activation of both the CSP and a mycorrhizal-specific pathway downstream of the CSP (Oláh et al. 2005). Similarly, nonpurified diffusible AM fungal factors induce CSP-dependent gene expression (Kuhn et al. 2010; Weidmann et al. 2004), calcium oscillations (Chabaud et al. 2011; Kosuta et al. 2008), and activation of starch-related metabolism (Gutjahr et al. 2009).

Maillet and associates (2011) have now shown that pure Myc-LCO induce CSP-dependent gene expression and stimulation of root branching in *M. truncatula*. Interestingly, this latter response is DMI3-dependent, unlike other work that showed DMI3-independent stimulation of root branching in *M. truncatula* by nonpurified diffusible AM fungal factors (Mukherjee and Ané 2011; Oláh et al. 2005), suggesting the existence of more than one pathway. The new work has also revealed increased complexity in the intricacy of the Nod and Myc signaling pathways in *M. truncatula*, namely that *MtNSP2* now appears to be part of the CSP (Fig. 3). Thus, *Mtnsp2* mutant plants are blocked for Myc-LCO stimulation of root branching and show approximately 40% reduction in mycorrhization levels compared with wild-type plants (Maillet et al. 2011). Redundancy is a possible explanation for this mycorrhizal phenotype. Since it has been shown that the MtNSP2 protein forms a complex with MtNSP1 that then binds to promoter elements of genes involved in nodulation (Hirsch et al. 2009), it is likely that MtNSP2 is also a component of another DNA binding complex that controls expression of mycorrhization genes. In light of this, it is interesting that a *Ljnsp2* mutant has been complemented for nodulation by a *NSP2*-like gene from the nonlegume rice and that an amino acid important in MtNSP2 for the interaction with MtNSP1 is conserved in the rice NSP2 protein (Yokota et al. 2010).

The roles of calcium in symbiotic signaling. Nod factors and diffusible AM fungal factors both induce a calcium spiking response in host plant cells (Chabaud et al. 2011; Kosuta et al. 2008; Oldroyd and Downie 2006; Sieberer et al. 2009). Nod factors also induce a rapid calcium flux that requires higher concentrations of Nod factors than calcium spiking and is still induced in early CSP mutants (Shaw and Long 2003). AM fungal–induced calcium spiking has been detected in legume and nonlegume plants (Chabaud et al. 2011; Kosuta et al. 2008). Nod factor–induced and AM fungal–induced calcium spiking responses are quite similar, but mycorrhizal calcium spiking is less regular than Nod factor–induced spiking (Chabaud et al. 2011; Kosuta et al. 2008). Much evidence supports an essential role of calcium spiking in Nod signaling leading to nodulation and Myc signaling leading to mycorrhization (Oldroyd and Downie 2006). In particular, all plant mutants that are deficient for calcium spiking—induced by either Nod factors or AM fungi—are also symbiotically-deficient. In legumes, an autoactive form of the CCaMK is sufficient for nodule organogenesis (Gleason et al. 2006; Tirichine et al. 2006) and can complement *L. japonicus* calcium spiking–deficient mutants for nodulation and mycorrhization (Hayashi et al. 2010; Madsen et al. 2010), indicating that early CSP signaling components function to activate CCaMK via calcium spiking.

Now, it is important to test calcium spiking induced by Myc-LCO and compare this with Nod factor–induced spiking. Chabaud and associates (2011) suggested that the different characteristics of the calcium spiking they reported in *M. truncatula* by spore exudates compared with hyphopodium contact

could indicate that the fungal signals produced by the presymbiotic mycelium differ from those generated following hyphopodium formation on the root surface. The nature of these signals clearly needs to be investigated. Interestingly, a *M. truncatula* CCaMK mutant can be complemented for nodulation by the orthologous gene from rice (Godfroy et al. 2006). Possible explanations for this may be that the calcium signatures and the CCaMK downstream targets are similar in the AM and RL symbioses or that the Nod and Myc calcium signatures are different but only the CCaMK of legume plants can differentiate them. Other nonlegume genes can also propagate a Nod factor–induced signal, since NSP-like genes from tobacco and rice can complement legume *nsp* mutants for nodulation (Heckmann et al. 2006; Yokota et al. 2010).

Specificity and regulation of symbiotic signaling. If differences in calcium spiking signatures are not sufficient to activate specific Nod and Myc signaling pathways downstream of the CSP in legume plants, there must be one or more additional parallel signaling pathways by which the plant distinguishes symbionts. This is suggested by complementation experiments with a gain-of-function version of CCaMK that can restore nodulation but not rhizobial infection to *L. japonicus* Nod factor receptor mutants (Hayashi et al. 2010; Madsen et al. 2010). The rapid calcium flux could be involved in a CSP-independent and Nod-specific infection signaling pathway (Kouchi et al. 2010), and CCaMK is likely to play a key role in cross-signaling and coordination between the CSP and such a rhizobial infection pathway (Hayashi et al. 2010; Madsen et al. 2010). In *L. japonicus* NAP1, PIR1, and CERBERUS are likely candidates to control this infection pathway (Yano et al. 2009; Yokota et al. 2009), while in *M. truncatula*, MtLYK3, LIN, and RPG are likely to be involved (Arrighi et al. 2008; Kiss et al. 2009; Limpens et al. 2003; Smit et al. 2007). MtNFP must also be involved in controlling infection, in addi-

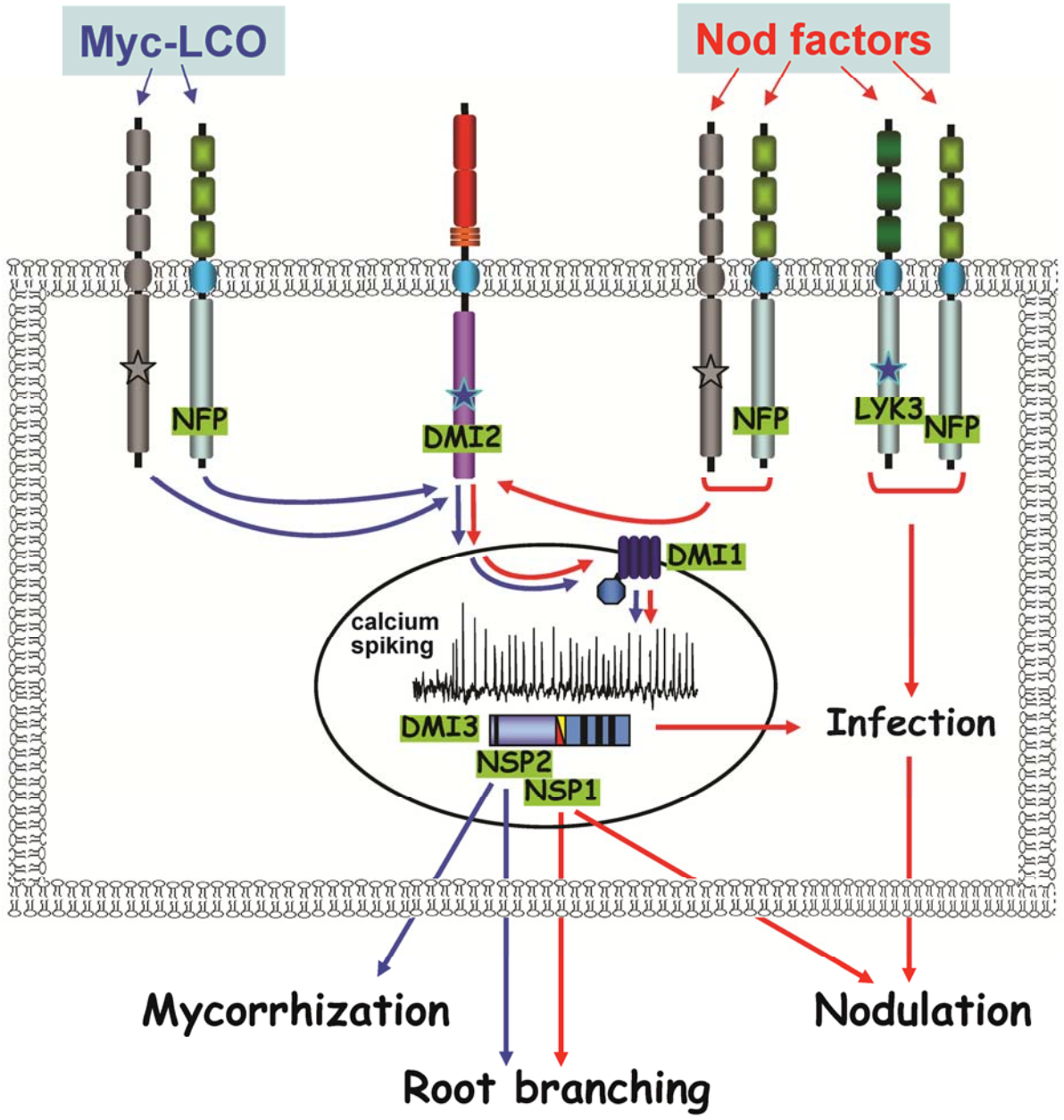

Fig. 3. Model for Nod factor and mycorrhizal lipo-chitooligosaccharide (Myc-LCO) perception and early signaling in *Medicago truncatula*. Lysin-motif (LysM)-RLK proteins as well as the DMI1, DMI2, and DMI3 proteins are schematically represented. Stars represent active kinase domains. As-yet-unknown LysM-RLK proteins with active kinase domains are postulated to act as components of a Myc-LCO receptor and of a Nod factor receptor. Note that the calcium-spiking profiles may be different for Nod factor and Myc-LCO signaling.

tion to its role in controlling the CSP (Arrighi et al. 2006; S. Bensmihen, *personal communication*), and is likely to act together with MtLYK3 to both control the high level of symbiont-host specificity that characterizes the RL symbiosis and, at the same time, contribute to the differentiation between Nod and Myc-LCO. It will be interesting to have Myc-LCO receptor mutants and transform them with a gain-of-function version of CCaMK to see if this is sufficient for mycorrhizal infection. This would give insights into whether there is also a parallel signaling pathway involved specifically in mycorrhizal infection.

MtNSP1 and MtRAM1 are components of Nod and Myc-specific pathways, respectively, downstream of the CSP. Thus, *Mtnsp1* mutants only respond to Myc-LCO and are Nod-Myc+, while *Mtram1* mutants only respond to Nod factors and are Nod+Myc– (Maillet et al. 2011; Oláh et al. 2005; G. Oldroyd, *personal communication*). This is consistent with studies that have revealed little overlap between genes differentially expressed during nodulation and mycorrhization (Hohnjec et al. 2005). Moreover, downstream of the CSP, only the Nod pathway leads to nodule organogenesis involving cortical cell division and de novo meristem formation. This involves articulation with cytokinin signaling and a symbiotic protein called NIN (Frugier et al. 2008). In contrast, the stimulation of root branching, which is likely to involve auxin signaling, is induced by both specific pathways. This could either be due to convergence of the pathways downstream of MtNSP1 and MtRAM1 or involve different mechanisms. The ability of rhizobia to alter the root auxin balance is also a prerequisite for nodule formation, with differing ratios of auxin and cytokinin being likely to specify the precursors of the different root organs—nodules and lateral roots—in legume plants (Mathesius 2008).

The control of the tissue and subcellular localization of receptors and signaling proteins is likely to contribute to the specificity of Nod and Myc responses. In *M. truncatula*, Nod factor signaling is active throughout infection and even during rhizobial release into host cells of nodules (Arrighi et al. 2006; Bersoult et al. 2005; Limpens et al. 2005), while work in *L. japonicus* suggests that different signaling mechanisms may operate in root-epidermal, as compared with nodule-cortical, tissue (Groth et al. 2010; Madsen et al. 2010). Interestingly, although Nod factors and AM fungi both induce calcium spiking in root-hair cells, recent work indicates that calcium spiking induced by AM fungal spore exudates is detected best in non-root hair cells (Chabaud et al. 2011), suggesting that this latter cell type is most responsive to AM fungi. For mycorrhization, the fact that CSP mutants can form hyphopodia but are deficient for eliciting PPA formation in epidermal cells (Genre et al. 2005) suggests that there are important local signaling mechanisms. At the subcellular level, a remorin protein (Lefebvre et al. 2010) and two flotillin proteins (Haney and Long 2010), which are induced during the RL symbiosis and required for rhizobial infection, have been shown to be localized in plasma-membrane microdomains in *M. truncatula*. As the remorin interacts with symbiotic receptors (Lefebvre et al. 2010), these plasma-membrane microdomains are implicated in a mechanism of positional Nod factor signaling during infection. It should also be noted that a *L. japonicus* remorin gene is strongly induced during the AM symbiosis (Kistner et al. 2005).

Other characterized responses indicate that there are common regulatory elements in nodulation and mycorrhization. For example, Nod factors and Myc-LCO both stimulate mycorrhization (Maillet et al. 2011; Oláh et al. 2005; Xie et al. 1995, 1998). Another example is the cross-induction of autoregulation, whereby nodulation of one part of a root system of alfalfa suppresses both further nodulation and mycorrhization of other roots (Catford et al. 2003). Similarly, alfalfa root systems precolonized on one side by AM fungi exhibit reduced nodule formation on the other side (Catford et al. 2003). Systemically induced changes in flavonoid levels are implicated in these effects (Catford et al. 2006), consistent with the critical role played by flavonoids in nodulation (J. Zhang et al. 2009) and implicating flavonoids in a mechanism by which mycorrhization is controlled. Also, several hypernodulation mutants are hyperinfected by AM fungi (Oka-Kira and Kawaguchi 2006). A plant gene that is involved in controlling both nodulation and mycorrhization is *ENOD40*, which is rapidly induced in the pericycle and dividing cells of nodule primordia in *Medicago* spp. (Compaan et al. 2001). Knock-down of ENOD40 results in reduced nodulation and reduced mycorrhization, and overexpression of ENOD40 results in accelerated nodulation and increased mycorrhization (Charon et al. 1999; Staehelin et al. 2001). These effects might involve hormonal changes, such as cytokinin, which, together with auxin transport inhibitors, are connected to activation and action of ENOD40.

Cross-talk and specificity in symbiotic and defense signaling.

The role of structurally related signals and LysM proteins in symbiotic and defense responses raises the question of how specificity of perception and signal transduction is achieved. Cross-talk between CO and LCO signaling is suggested, since CO can induce both CSP-dependent calcium spiking in *M. truncatula* and *L. japonicus* (Oldroyd et al. 2001; Walker et al. 2000) and CSP-dependent expression of the symbiotic transcription factor genes *LjNIN*, *LjNSP1*, and *LjNSP2* (Nakagawa et al. 2011). This latter response is independent of LjNFR1, suggesting that a CO receptor can cross-activate the CSP. The functional similarities and differences between the kinase domains of the *Arabidopsis* CO receptor CERK1 and the closely-related Nod factor receptor LjNFR1 have recently been shown by domain-swap experiments. Although the kinase domain of CERK1 will not substitute for the corresponding region of LjNFR1 in symbiotic signaling, only small changes in specific regions confer on it the ability to transduce the Nod factor signal for symbiosis in combination with the LjNFR1 extracellular region (Nakagawa et al. 2011). There is also evidence that Nod factors may activate defense signaling pathways, as Nod factors induce LjNFR1-dependent expression of some defense-gene homologs in *L. japonicus* (Nakagawa et al. 2011) and a cell-death response is induced in *Nicotiana benthamiana* leaves when Nod factor receptor pairs are overexpressed (Madsen et al. 2011; A. Pietraszeska-Bogiel, B. Lefebvre, J. Cullimore, and T. Cadella, *unpublished data*). However, induction of many defense genes by Nod factors and during symbiosis is either transient or absent (El Yahyaoui et al. 2004; Hogslund et al. 2009; Lohar et al. 2006; Nakagawa et al. 2011), and defense-like reactions could be confined to specific cells, such as when a hypersensitive reaction is associated with aborted rhizobial infection events in *Medicago sativa* (Vasse et al. 1993). Further insights into how plants achieve specificity in symbiotic or defense response activation by LCO and CO should be achieved once legume CO receptors are identified.

Intriguingly, although AM fungi are symbiotic microorganisms, they still have chitin-rich cell walls, raising the question of how they avoid defense induction by this chitin. The production by fungi of chitin deacetylases might contribute to this escape, as the end product chitosan is much less active in defense induction than chitin. Another strategy that involves LysM proteins has been deciphered for a non-AM fungus, *Cladosporium fulvum*, but might, none-the-less, be a widespread

phenomenon among diverse fungi (de Jonge and Thomma 2009). In *C. fulvum*, the LysM protein Ecp6 has been shown to sequester CO to prevent defense induction (de Jonge et al. 2010).

The evolution of symbiotic signaling: recruitment and acquisition.

The AM symbiosis is very widespread among plants, and fossil evidence from approximately 400 million years ago found arbuscules inside the first land plants (Redecker et al. 2000; Remy et al. 1994). Moreover, mycorrhizal genes were present in the common ancestors of land plants, and *CCaMK* orthologs from liverworts and hornworts can complement *M. truncatula CCaMK* mutants for mycorrhization (Wang et al. 2010). Since the AM symbiosis is considered to be one of the key processes that contributed to the origin of land flora (Humphreys et al. 2010), LCO may have been major actors in evolution. Legumes evolved much later (about 60 million years ago) (Sprent 2007), followed by the appearance of nodulation, which probably involved certain diazotrophic bacteria acquiring the ability to synthesize LCO by horizontal gene transfer. These LCO biosynthetic genes are located on plasmids or in symbiotic islands and have an evolutionary history different from that of chromosomal genes (Masson-Boivin et al. 2009). Rhizobia would then have been able to activate part of the plant Myc signaling pathway (the CSP), using the LCO they produced.

The new results implicating MtNFP in Myc-LCO perception (Maillet et al. 2011) suggest that the RL symbiosis recruited this LYR-type LysM-RLK along with the CSP. This is supported by the finding that a single *NFP*-like gene in *Parasponia andersonii* controls the establishment of the RL and the AM symbioses, and the ability of this nonlegume plant to form root nodules with rhizobia is probably at least partly due to the recruitment of this mycorrhizal LysM protein (Op den Camp et al. 2010). Furthermore, Op den Camp and associates (2010) suggest that the *NFP* gene was duplicated in many legumes during evolution and one copy was maintained for the AM symbiosis, while the other copy evolved over time to recognize precise Nod-LCO signals to allow nodulation with specific rhizobia. This adaptation to recognize Nod factors probably did not involve loss of kinase activity, since the *Parasponia NFP*-like gene is predicted to encode a protein with a degenerate intracellular region devoid of kinase activity and similar genes are found in diverse plant species including apple, monocots, and the primitive plant *Selaginella moellendorfi* (Arrighi et al. 2006; Op den Camp et al. 2010; X. C. Zhang et al. 2009) but is more likely to result from changes to the extracellular LysM domains, particularly LysM2 (Radutoiu et al. 2007; S. Bensmihen, *personal communication*).

Legume evolution to specifically control Nod factor recognition for rhizobial infection also involved the appearance of a second Nod factor receptor protein, MtLYK3/LjNFR1, probably also as the result of gene duplication and neofunctionalization in the LYK gene subfamily (X. C. Zhang et al. 2009). Compared with MtNFP/LjNFR5, this second LysM-RLK is phylogenetically much closer to the *A. thaliana* chitin receptor CERK1, and there is microsynteny between the genomic regions around *MtLYK3*, *LjNFR1*, and *AtCERK1*, suggesting that they are descendents of a common ancestor (Zhu et al. 2006). Here again, the extracellular LysM domains have evolved to recognize specific structures of LCO, since a chimeric construct consisting of the extracellular region of LjNFR1 combined with the intracellular kinase domain of MtLYK3 can complement a *Ljnfr1* mutant for nodulation by *Mesorhizobium loti*, whereas the *MtLYK3* gene cannot (Nakagawa et al. 2011). It will be interesting to find out whether a LYK-type protein is also involved in a Myc-LCO receptor complex. In addition to evolutionary changes linked to Nod factor recognition, specific evolution of the CSP gene *MtDMI2/LjSYMRK* has also occurred in plants able to nodulate. Thus, Markmann and associates (2008) identified three different structural versions of this gene among diverse plants, and the shorter forms present in the nonlegumes rice and tomato were sufficient to complement a legume mutant for mycorrhization but not for nodulation.

Since the acquisition of LCO production by bacteria, notable evolutionary changes include the induction of Nod factor synthesis by host plant flavonoids and the appearance of a great diversity of Nod factor structures. Evidence suggests that rhizobia have evolved in the centers of legume origin and diversification such that coevolution would explain the recognition of host flavonoids and the high level of Nod factor structural variation, both of which contribute largely to symbiont-partner specificity (Martinez-Romero 2009). Simultaneously, legume LysM-RLK proteins have evolved so that legumes can distinguish both between Nod-LCO and Myc LCO (rhizobial mutants producing Nod factors structurally very similar to Myc-LCO are deficient for symbiosis) and among precise Nod-LCO structures (most legume plants can only be nodulated by rhizobia producing a certain Nod factor structure). The much wider host range of AM fungi and their limited genetic diversity (Redecker et al. 2000), suggests that there could be limited variation in structure of Myc-LCO among different AM fungi.

The dogma that all rhizobia produce Nod factors changed in 2007, with the finding that some photosynthetic *Bradyrhizobium* species do not contain *nod* genes (Giraud et al. 2007). This characteristic has recently been shown to be shared by all cross-inoculation group 3 strains of bradyrhizobia that nodulate tropical legumes of the genus *Aeschynomene* (Miche et al. 2010), and could be explained either if *nod* genes were lost in certain cases or if they were late acquisitions for nodule formation. As for Nod factor–producing rhizobia, the nodulation process of such Nod factor–deficient rhizobia is likely to involve cytokinin signaling (Bonaldi et al. 2010), but it is not yet known whether the CSP is involved. For infection, this is entirely intercellular by crack-entry, which, together with recent work in *L. japonicus* in which Nod factor–independent intercellular infection was also described (Madsen et al. 2010), indicates that the ability of rhizobia to produce Nod factors can confer on them the ability to infect via infection threads.

An alternative mechanism to activate the CSP might have evolved for the actinorhizal symbiosis. In actinorhizal plants, the CSP gene *MtDMI2/LjSYMRK* controls both nodulation by *Frankia* spp. and mycorrhization (Gherbi et al. 2008; Markmann et al. 2008), and so far, attempts to purify LCO from active supernatants of *Frankia* spp. have failed (Cérémonie et al. 1999). Furthermore, although genes homologous to *nodB*, *nodC*, *nodD*, and *nodQ* (Nod factor synthesis genes) are found in *Frankia* genomes, they have low levels of sequence identity as compared with rhizobial genes and are not organized in clusters or operons, as in rhizobia (Normand et al. 2007). Moreover, a recent transcriptome study showed that these genes are not differentially expressed between free-living and symbiotic conditions (Alloisio et al. 2010), leaving open questions about the role of these genes in symbiosis and whether there is a non-LCO signal for activating the CSP for nodulation by *Frankia* spp.

Concluding remarks and outstanding questions.

The identification of Myc-LCO 20 years after Nod factors were discovered is a breakthrough in the study of symbiotic plant-microbe interactions. The structural similarity of Myc-LCO to Nod factors suggests that Myc-LCO could play an analogous role in mycorrhization as Nod factors in nodulation.

Myc-LCO are likely to be important for symbiosis as they stimulate mycorrhization by the AM fungus in various host plants, and in *M. truncatula*, they stimulate an increase in the number of lateral roots, which are preferential sites for infection by AM fungi. However, whereas rhizobial genetics proved the importance of Nod factors in nodulation, the coenocytic and obligate biotrophic nature of AM fungi has made it impossible to use microbial genetics to determine if the newly identified Myc-LCO signals are required for mycorrhization. The development of strategies to produce Myc-LCO mimetics (Maillet et al. 2011) will facilitate further studies aimed at both evaluating the importance of these molecules in mycorrhization and determining their mechanisms of perception and signal transduction. The identification of more mycorrhization-deficient plant mutants and the analysis of other mycorrhization responses would lead to a better understanding of the activity of Myc-LCO and of other types of mycorrhizal signals. In this respect, it will be particularly important to use Myc-LCO to characterize calcium spiking and specific mycorrhizal transcriptional responses.

The structure of Myc-LCO and the observation that a *Parasponia* LysM-RLK gene is required for both nodulation and mycorrhization suggests that members of the LysM family should be assessed in other plants for their roles in mycorrhization and Myc-LCO responses. A major objective of this would be to identify one or more Myc-LCO receptors and assess their roles in mycorrhization. The *Parasponia* PaNFP receptor should also be evaluated for its role in Myc-LCO responses. Clearly studies on legumes should be extended to other higher plants in which there is no overlap with Nod factor signaling. Together, this work would open the way to further understand the specificities and cross-talk in Myc-LCO and Nod factor signaling in legumes (and *Parasponia* spp.) and between LCO and CO signaling in many plants.

On the microbial side, the structure of Myc-LCO should be determined from different AM fungi. Coupled with studies on the specificity of receptors, this work would help to understand the broad host range of AM fungi and whether the production of sulphated and nonsulphated Myc-LCO is important for the structural preference of different host plants. The genome of *G. intraradices* is currently being sequenced, and data mining on this and other AM fungal genomes should enable the genes encoding the synthesis of Myc-LCO to be identified. Studies on gene activation and Myc-LCO production would establish whether strigolactones, which induce preinfection branching and metabolic activity of AM fungi (Akiyama et al. 2005; Besserer et al. 2006; Xie et al. 2010), can also stimulate Myc-LCO production, in a way analogous to the activation of *nod* gene expression and Nod factor production by plant flavonoids. Studies on the phylogeny, regulation, and biochemical function of such genes would also help our understanding of both host range and the role of Myc-LCO during various stages of symbiotic signaling. Finally, searching for homologous genes among other microorganisms would provide a means of exploring whether LCO signals could be more widespread in plant-microbe interactions.

ACKNOWLEDGMENTS

We are grateful to S. Bensmihen, C. Rosenberg, B. Lefebvre, C. Hervé, F. Debellé, J.-J. Bono, J. Dénarié, and G. Bécard for helpful discussions and for critically reading the manuscript. We apologize to people whose work we have not cited because of length constraints. We thank V. Poinsot for help with Figure 1. We acknowledge funding of recent work on LCO signaling in our group by the Agence Nationale de la Recherche (contracts NodBindsLysM, SYMPASIGNAL, and MycSignalling) and the Marie Curie Actions of the European Community (contract NODPERCEPTION).

LITERATURE CITED

Akiyama, K., Matsuzaki, K., and Hayashi, H. 2005. Plant sesquiterpenes induce hyphal branching in arbuscular mycorrhizal fungi. Nature 435:824-827.

Alloisio, N., Queiroux, C., Fournier, P., Pujic, P., Normand, P., Vallenet, D., Medigue, C., Yamaura, M., Kakoi, K., and Kucho, K. 2010. The *Frankia alni* symbiotic transcriptome. Mol. Plant-Microbe Interact. 23:593-607.

Ardourel, M., Demont, N., Debellé, F., Maillet, F., de Billy, F., Promé, J. C., Dénarié, J., and Truchet, G. 1994. *Rhizobium meliloti* lipooligosaccharide nodulation factors: Different structural requirements for bacterial entry into target root hair cells and induction of plant symbiotic developmental responses. Plant Cell 6:1357-1374.

Arrighi, J. F., Barre, A., Ben Amor, B., Bersoult, A., Soriano, L. C., Mirabella, R., de Carvalho-Niebel, F., Journet, E. P., Gherardi, M., Huguet, T., Geurts, R., Dénarié, J., Rougé, P., and Gough, C. 2006. The *Medicago truncatula* lysin motif-receptor-like kinase gene family includes NFP and new nodule-expressed genes. Plant Physiol. 142:265-279.

Arrighi, J. F., Godfroy, O., de Billy, F., Saurat, O., Jauneau, A., and Gough, C. 2008. The RPG gene of *Medicago truncatula* controls *Rhizobium*-directed polar growth during infection. Proc. Natl. Acad. Sci. U.S.A. 105:9817-9822.

Banba, M., Gutjahr, C., Miyao, A., Hirochika, H., Paszkowski, U., Kouchi, H., and Imaizumi-Anraku, H. 2008. Divergence of evolutionary ways among common *sym* genes: CASTOR and CCaMK show functional conservation between two symbiosis systems and constitute the root of a common signaling pathway. Plant and Cell Physiol. 49:1659-1671.

Bek, A. S., Sauer, J., Thygesen, M. B., Duus, J. O., Petersen, B. O., Thirup, S., James, E., Jensen, K. J., Stougaard, J., and Radutoiu, S. 2010. Improved characterization of nod factors and genetically based variation in LysM Receptor domains identify amino acids expendable for nod factor recognition in *Lotus* spp. Mol. Plant-Microbe Interact. 23:58-66.

Ben Amor, B., Shaw, S. L., Oldroyd, G. E. D., Maillet, F., Penmetsa, R. V., Cook, D., Long, S. R., Dénarié, J., and Gough, C. 2003. The NFP locus of *Medicago truncatula* controls an early step of Nod factor signal transduction upstream of a rapid calcium flux and root hair deformation. Plant J. 34:495-506.

Bersoult, A., Camut, S., Perhald, A., Kereszt, A., Kiss, G. B., and Cullimore, J. V. 2005. Expression of the *Medicago truncatula DMI2* gene suggests roles of the symbiotic nodulation receptor kinase in nodules and during early nodule development. Mol. Plant-Microbe Interact. 18:869-876.

Besserer, A., Puech-Pages, V., Kiefer, P., Gomez-Roldan, V., Jauneau, A., Roy, S., Portais, J. C., Roux, C., Becard, G., and Sejalon-Delmas, N. 2006. Strigolactones stimulate arbuscular mycorrhizal fungi by activating mitochondria. PLoS. Biol. 4:1239-1247.

Boller, T., and Felix, G. 2009. A renaissance of elicitors: Perception of microbe-associated molecular patterns and danger signals by pattern-recognition receptors. Annu. Rev. Plant Biol. 60:379-406.

Bonaldi, K., Gourion, B., Fardoux, J., Hannibal, L., Cartieaux, F., Boursot, M., Vallenet, D., Chaintreuil, C., Prin, Y., Nouwen, N., and Giraud, E. 2010. Large-scale transposon mutagenesis of photosynthetic *Bradyrhizobium* sp strain ORS278 reveals new genetic loci putatively important for Nod-independent symbiosis with *Aeschynomene indica*. Mol. Plant-Microbe Interact. 23:760-770.

Bonfante, P., and Genre, A. 2010. Mechanisms underlying beneficial plant-fungus interactions in mycorrhizal symbiosis. Nat. Commun. Jul 27;1:48. doi: 10.1038/ncomms1046. Published online.

Bono, J. J., Riond, J., Nicolaou, K. C., Bockovich, N. J., Estevez, V. A., Cullimore, J. V., and Ranjeva, R. 1995. Characterization of a binding site for chemically synthesized lipo-oligosaccharidic NodRm factors in particulate fractions prepared from roots. Plant J. 7:253-260.

Buist, G., Steen, A., Kok, J., and Kuipers, O. P. 2008. LysM, a widely distributed protein motif for binding to (peptido)glycans. Mol. Microbiol. 68:838-847.

Catford, J. G., Staehelin, C., Lerat, S., Piche, Y., and Vierheilig, H. 2003. Suppression of arbuscular mycorrhizal colonization and nodulation in split-root systems of alfalfa after pre-inoculation and treatment with Nod factors. J. Exp. Bot. 54:1481-1487.

Catford, J. G., Staehelin, C., Larose, G., Piche, Y., and Vierheilig, H. 2006. Systemically suppressed isoflavonoids and their stimulating effects on nodulation and mycorrhization in alfalfa split-root systems. Plant Soil 285:257-266.

Catoira, R., Galera, C., de Billy, F., Penmetsa, R. V., Journet, E. P., Maillet, F., Rosenberg, C., Cook, D., Gough, C., and Dénarié, J. 2000. Four genes of *Medicago truncatula* controlling components of a Nod factor transduction pathway. Plant Cell 12:1647-1666.

Catoira, R., Timmers, A. C., Maillet, F., Galera, C., Penmetsa, R. V., Cook, D., Dénarié, J., and Gough, C. 2001. The *HCL* gene of *Medicago trun-*

catula controls *Rhizobium*-induced root hair curling. Development 128:1507-1518.

Cérémonie, H., Debellé, F., and Fernandez, M. P. 1999. Structural and functional comparison of *Frankia* root hair deforming factor and rhizobia Nod factor. Can. J. Bot. 77:1293-1301.

Chabaud, M., Genre, A., Sieberer, B. J., Faccio, A., Fournier, J., Novero, M., Barker, D. G., and Bonfante, P. 2011. Arbuscular mycorrhizal hyphopodia and germinated spore exudates trigger Ca^{2+} spiking in the legume and nonlegume root epidermis. New Phytol. 189:347-355.

Charon, C., Sousa, C., Crespi, M., and Kondorosi, A. 1999. Alteration of enod40 expression modifies *Medicago truncatula* root nodule development induced by *Sinorhizobium meliloti*. Plant Cell 11:1953-1965.

Chen, C. Y., Gao, M. Q., Liu, J. Y., and Zhu, H. Y. 2007. Fungal symbiosis in rice requires an ortholog of a legume common symbiosis gene encoding a Ca^{2+}/calmodulin-dependent protein kinase. Plant Physiol. 145:1619-1628.

Chen, C. Y., Ané, J. M., and Zhu, H. Y. 2008. OsIPD3, an ortholog of the *Medicago truncatula* DMI3 interacting protein IPD3, is required for mycorrhizal symbiosis in rice. New Phytol. 180:311-315.

Chen, C. Y., Fan, C., Gao, M. Q., and Zhu, H. Y. 2009. Antiquity and function of CASTOR and POLLUX, the twin ion channel-encoding genes key to the evolution of root symbioses in plants. Plant Physiol. 149:306-317.

Compaan, B., Yang, W. C., Bisseling, T., and Franssen, H. 2001. ENOD40 expression in the pericycle precedes cortical cell division in Rhizobium-legume interaction and the highly conserved internal region of the gene does not encode a peptide. Plant Soil 230:1-8.

D'Haeze, W., and Holsters, M. 2002. Nod factor structures, responses, and perception during initiation of nodule development. Glycobiol. 12:79R-105R.

de Jonge, R., and Thomma, B. 2009. Fungal LysM effectors: extinguishers of host immunity? Trends Microbiol. 17:151-157.

de Jonge, R., van Esse, H. P., Kombrink, A., Shinya, T., Desaki, Y., Bours, R., van der Krol, S., Shibuya, N., Joosten, M. H., and Thomma, B. P. 2010. Conserved fungal LysM effector Ecp6 prevents chitin-triggered immunity in plants. Science 329:953-955.

Dénarié, J., Debellé, F., and Promé, J. C. 1996. Rhizobium lipo-chitooligosaccharide nodulation factors: Signaling molecules mediating recognition and morphogenesis. Annu Rev Biochem 65:503-535.

El Yahyaoui, F., Kuster, H., Ben Amor, B., Hohnjec, N., Puhler, A., Becker, A., Gouzy, J., Vernie, T., Gough, C., Niebel, A., Godiard, L., and Gamas, P. 2004. Expression profiling in *Medicago truncatula* identifies more than 750 genes differentially expressed during nodulation, including many potential regulators of the symbiotic program. Plant Physiol. 136:3159-3176.

Etzler, M. E., Kalsi, G., Ewing, N. N., Roberts, N. J., Day, R. B., and Murphy, J. B. 1999. A nod factor binding lectin with apyrase activity from legume roots. Proc. Natl. Acad. Sci. U.S.A. 96:5856-5861.

Ferguson, B. J., Indrasumunar, A., Hayashi, S., Lin, M. H., Lin, Y. H., Reid, D. E., and Gresshoff, P. M. 2010. Molecular analysis of legume nodule development and autoregulation. J. Integr. Plant Biol. 52:61-76.

Frugier, F., Kosuta, S., Murray, J. D., Crespi, M., and Szczyglowski, K. 2008. Cytokinin: Secret agent of symbiosis. Trends Plant Sci. 13:115-120.

Genre, A., Chabaud, M., Timmers, T., Bonfante, P., and Barker, D. G. 2005. Arbuscular mycorrhizal fungi elicit a novel intracellular apparatus in *Medicago truncatula* root epidermal cells before infection. Plant Cell 17:3489-3499.

Gherbi, H., Markmann, K., Svistoonoff, S., Estevan, J., Autran, D., Giczey, G., Auguy, F., Peret, B., Laplaze, L., Franche, C., Parniske, M., and Bogusz, D. 2008. SymRK defines a common genetic basis for plant root endosymbioses with arbuscular mycorrhiza fungi, rhizobia, and Frankia bacteria. Proc. Natl. Acad. Sci. U.S.A. 105:4928-4932.

Giraud, E., Moulin, L., Vallenet, D., Barbe, V., Cytryn, E., Avarre, J. C., Jaubert, M., Simon, D., Cartieaux, F., Prin, Y., Bena, G., Hannibal, L., Fardoux, J., Kojadinovic, M., Vuillet, L., Lajus, A., Cruveiller, S., Rouy, Z., Mangenot, S., Segurens, B., Dossat, C., Franck, W. L., Chang, W. S., Saunders, E., Bruce, D., Richardson, P., Normand, P., Dreyfus, B., Pignol, D., Stacey, G., Emerich, D., Vermeglio, A., Medigue, C., and Sadowsky, M. 2007. Legumes symbioses: Absence of nod genes in photosynthetic bradyrhizobia. Science 316:1307-1312.

Gleason, C., Chaudhuri, S., Yang, T., Munoz, A., Poovaiah, B. W., and Oldroyd, G. E. 2006. Nodulation independent of rhizobia induced by a calcium-activated kinase lacking autoinhibition. Nature 441:1149-1152.

Godfroy, O., Debellé, F., Timmers, T., and Rosenberg, C. 2006. A rice calcium- and calmodulin-dependent protein kinase restores nodulation to a legume mutant. Mol. Plant-Microbe Interact. 19:495-501.

Goedhart, J., Rohrig, H., Hink, M. A., van Hoek, A., Visser, A. J., Bisseling, T., and Gadella, T. W., Jr. 1999. Nod factors integrate spontaneously in biomembranes and transfer rapidly between membranes and to root hairs, but transbilayer flip-flop does not occur. Biochemistry 38:10898-10907.

Gomez, S. K., Javot, H., Deewatthanawong, P., Torres-Jerez, I., Tang, Y. H., Blancaflor, E. B., Udvardi, M. K., and Harrison, M. J. 2009. *Medicago truncatula* and *Glomus intraradices* gene expression in cortical cells harboring arbuscules in the arbuscular mycorrhizal symbiosis. BMC Plant Biol. 9:10 doi:10.1186/1471-2229-9-10. Published online.

Gonzalez, L., Bernabe, M., Espinosa, J. F., Tejero-Mateo, P., Gil-Serrano, A., Mantegazza, N., Imberty, A., Driguez, H., and Jimenez-Barbero, J. 1999. Solvent-dependent conformational behaviour of lipochitoligosaccharides related to Nod factors. Carbohydr. Res. 318:10-19.

Goormachtig, S., Capoen, W., and Holsters, M. 2004. Rhizobium infection: Lessons from the versatile nodulation behaviour of water-tolerant legumes. Trends Plant Sci. 9:518-522.

Govindarajulu, M., Kim, S. Y., Libault, M., Berg, R. H., Tanaka, K., Stacey, G., and Taylor, C. G. 2009. GS52 ecto-apyrase plays a critical role during soybean nodulation. Plant Physiol. 149:994-1004.

Gressent, F., Drouillard, S., Mantegazza, N., Samain, E., Geremia, R. A., Canut, H., Niebel, A., Driguez, H., Ranjeva, R., Cullimore, J., and Bono, J. J. 1999. Ligand specificity of a high-affinity binding site for lipo-chitooligosaccharidic Nod factors in *Medicago* cell suspension cultures. Proc. Natl. Acad. Sci. U.S.A. 96:4704-4709.

Groth, M., Takeda, N., Perry, J., Uchida, H., Draxl, S., Brachmann, A., Sato, S., Tabata, S., Kawaguchi, M., Wang, T. L., and Parniske, M. 2010. NENA, a *Lotus japonicus* homolog of Sec13, is required for rhizodermal infection by arbuscular mycorrhiza fungi and Rhizobia but dispensable for cortical endosymbiotic development. Plant Cell 22:2509-2526.

Groves, P., Offermann, S., Rasmussen, M. O., Canada, F. J., Bono, J. J., Driguez, H., Imberty, A., and Jimenez-Barbero, J. 2005. The relative orientation of the lipid and carbohydrate moieties of lipochitooligosaccharides related to nodulation factors depends on lipid chain saturation. Org. Biomol. Chem. 3:1381-1386.

Gutjahr, C., Banba, M., Croset, V., An, K., Miyao, A., An, G., Hirochika, H., Imaizumi-Anraku, H., and Paszkowski, U. 2008. Arbuscular mycorrhiza-specific signaling in rice transcends the common symbiosis signaling pathway. Plant Cell 20:2989-3005.

Gutjahr, C., Novero, M., Guether, M., Montanari, O., Udvardi, M., and Bonfante, P. 2009. Presymbiotic factors released by the arbuscular mycorrhizal fungus *Gigaspora margarita* induce starch accumulation in *Lotus japonicus* roots. New Phytol. 183:53-61.

Hamel, L. P., and Beaudoin, N. 2010. Chitooligosaccharide sensing and downstream signaling: Contrasted outcomes in pathogenic and beneficial plant-microbe interactions. Planta 232:787-806.

Haney, C. H., and Long, S. R. 2010. Plant flotillins are required for infection by nitrogen-fixing bacteria. Proc. Natl. Acad. Sci. U.S.A. 107:478-483.

Harrison, M. J. 2005. Signaling in the arbuscular mycorrhizal symbiosis. Annu. Rev. Microbiol. 59:19-42.

Hayashi, T., Banba, M., Shimoda, Y., Kouchi, H., Hayashi, M., and Imaizumi-Anraku, H. 2010. A dominant function of CCaMK in intracellular accommodation of bacterial and fungal endosymbionts. Plant J. 63:141-154.

Heckmann, A. B., Lombardo, F., Miwa, H., Perry, J. A., Bunnewell, S., Parniske, M., Wang, T. L., and Downie, J. A. 2006. *Lotus japonicus* nodulation requires two GRAS domain regulators, one of which is functionally conserved in a non-legume. Plant Physiol. 142:1739-1750.

Hirsch, S., Kim, J., Munoz, A., Heckmann, A. B., Downie, J. A., and Oldroyd, G. E. 2009. GRAS proteins form a DNA binding complex to induce gene expression during nodulation signaling in *Medicago truncatula*. Plant Cell 21:545-557.

Hogg, B. V., Cullimore, J. V., Ranjeva, R., and Bono, J. J. 2006. The *DMI1* and *DMI2* early symbiotic genes of *Medicago truncatula* are required for a high-affinity nodulation factor-binding site associated to a particulate fraction of roots. Plant Physiol. 140:365-373.

Hogslund, N., Radutoiu, S., Krusell, L., Voroshilova, V., Hannah, M. A., Goffard, N., Sanchez, D. H., Lippold, F., Ott, T., Sato, S., Tabata, S., Liboriussen, P., Lohmann, G. V., Schauser, L., Weiller, G. F., Udvardi, M. K., and Stougaard, J. 2009. Dissection of symbiosis and organ development by integrated transcriptome analysis of *Lotus japonicus* mutant and wild-type plants. PLoS One 4:e6556. Published online.

Hohnjec, N., Vieweg, M. F., Puhler, A., Becker, A., and Kuster, H. 2005. Overlaps in the transcriptional profiles of *Medicago truncatula* roots inoculated with two different *Glomus* fungi provide insights into the genetic program activated during arbuscular mycorrhiza. Plant Physiol. 137:1283-1301.

Humphreys, C. P., Franks, P. J., Rees, M., Bidartondo, M. I., Leake, J. R., and Beerling, D. J. 2010. Mutualistic mycorrhiza-like symbiosis in the most ancient group of land plants. Nat. Commun. 1:103. doi: 10.1038/ncomms1105. Published online.

Iizasa, E., Mitsutomi, M., and Nagano, Y. 2010. Direct binding of a plant LysM receptor-like kinase, LysM RLK1/CERK1, to chitin in vitro. J. Biol. Chem. 285:2996-3004.

Indrasumunar, A., Kereszt, A., Searle, I., Miyagi, M., Li, D. X., Nguyen, C. D. T., Men, A., Carroll, B. J., and Gresshoff, P. M. 2010. Inactivation of duplicated Nod Factor Receptor 5 (NFR5) genes in recessive loss-of-function non-nodulation mutants of allotetraploid soybean (*Glycine max* L. Merr.). Plant Cell Physiol. 51:201-214.

Indrasumunar, A., Searle, I., Lin, M. H., Kereszt, A., Men, A., Carroll, B. J., and Gresshoff, P. M. 2011. Nodulation factor receptor kinase 1alpha controls nodule organ number in soybean (*Glycine max* L. Merr). Plant J. 65:39-50.

Kaku, H., Nishizawa, Y., Ishii-Minami, N., Akimoto-Tomiyama, C., Dohmae, N., Takio, K., Minami, E., and Shibuya, N. 2006. Plant cells recognize chitin fragments for defense signaling through a plasma membrane receptor. Proc. Natl. Acad. Sci. U.S.A. 103:11086-11091.

Kalo, P., Gleason, C., Edwards, A., Marsh, J., Mitra, R. M., Hirsch, S., Jakab, J., Sims, S., Long, S. R., Rogers, J., Kiss, G. B., Downie, J. A., and Oldroyd, G. E. 2005. Nodulation signaling in legumes requires NSP2, a member of the GRAS family of transcriptional regulators. Science 308:1786-1789.

Kishimoto, K., Kouzai, Y., Kaku, H., Shibuya, N., Minami, E., and Nishizawa, Y. 2010. Perception of the chitin oligosaccharides contributes to disease resistance to blast fungus *Magnaporthe oryzae* in rice. Plant J. 64:343-354.

Kiss, E., Olah, B., Kalo, P., Morales, M., Heckmann, A. B., Borbola, A., Lozsa, A., Kontar, K., Middleton, P., Downie, J. A., Oldroyd, G. E. D., and Endre, G. 2009. LIN, a novel type of U-Box/WD40 protein, controls early infection by Rhizobia in legumes. Plant Physiol. 151:1239-1249.

Kistner, C., Winzer, T., Pitzschke, A., Mulder, L., Sato, S., Kaneko, T., Tabata, S., Sandal, N., Stougaard, J., Webb, K. J., Szczyglowski, K., and Parniske, M. 2005. Seven *Lotus japonicus* genes required for transcriptional reprogramming of the root during fungal and bacterial symbiosis. Plant Cell 17:2217-2229.

Klaus-Heisen, D., Nurisso, A., Pietraszewska-Bogiel, A., Mbengue, M., Camut, S., Timmers, T., Pichereaux, C., Rossignol, M., Gadella, T. W., Imberty, A., Lefebvre, B., and Cullimore, J. V. 2011. Structure-function similarities between a plant receptor-like kinase and the human interleukin-1 receptor-associated kinase-4. J. Biol. Chem. 286:11202-11210.

Kosuta, S., Chabaud, M., Lougnon, G., Gough, C., Dénarié, J., Barker, D. G., and Bécard, G. 2003. A diffusible factor from arbuscular mycorrhizal fungi induces symbiosis-specific *MtENOD11* expression in roots of *Medicago truncatula*. Plant Physiol. 131:952-962.

Kosuta, S., Hazledine, S., Sun, J., Miwa, H., Morris, R. J., Downie, J. A., and Oldroyd, G. E. D. 2008. Differential and chaotic calcium signatures in the symbiosis signaling pathway of legumes. Proc. Natl. Acad. Sci. U.S.A. 105:9823-9828.

Kouchi, H., Imaizumi-Anraku, H., Hayashi, M., Hakoyama, T., Nakagawa, T., Umehara, Y., Suganuma, N., and Kawaguchi, M. 2010. How many peas in a pod? Legume genes responsible for mutualistic symbioses underground. Plant Cell Physiol. 51:1381-1397.

Kucho, K., Hay, A. E., and Normand, P. 2010. The determinants of the actinorhizal symbiosis. Microbes Environ. 25:241-252.

Kuhn, H., Kuster, H., and Requena, N. 2010. Membrane steroid-binding protein 1 induced by a diffusible fungal signal is critical for mycorrhization in *Medicago truncatula*. New Phytol. 185:716-733.

Lefebvre, B., Timmers, T., Mbengue, M., Moreau, S., Hervé, C., Toth, K., Bittencourt-Silvestre, J., Klaus, D., Deslandes, L., Godiard, L., Murray, J. D., Udvardi, M. K., Raffaele, S., Mongrand, S., Cullimore, J., Gamas, P., Niebel, A., and Ott, T. 2010. A remorin protein interacts with symbiotic receptors and regulates bacterial infection. Proc. Natl. Acad. Sci. U.S.A. 107:2343-2348.

Lerouge, P., Roche, P., Faucher, C., Maillet, F., Truchet, G., Promé J.C., and Dénarié, J. 1990. Symbiotic host-specificity of *Rhizobium meliloti* is determined by a sulphated and acylated glucosamine oligosaccharide signal. Nature 344:781-784.

Limpens, E., Franken, C., Smit, P., Willemse, J., Bisseling, T., and Geurts, R. 2003. LysM domain receptor kinases regulating rhizobial Nod factor-induced infection. Science 302:630-633.

Limpens, E., Mirabella, R., Fedorova, E., Franken, C., Franssen, H., Bisseling, T., and Geurts, R. 2005. Formation of organelle-like N2-fixing symbiosomes in legume root nodules is controlled by DMI2. Proc. Natl. Acad. Sci. U.S.A. 102:10375-10380.

Lohar, D. P., Sharopova, N., Endre, G., Penuela, S., Samac, D., Town, C., Silverstein, K. A., and VandenBosch, K. A. 2006. Transcript analysis of early nodulation events in *Medicago truncatula*. Plant Physiol. 140:221-234.

Lohmann, G. V., Shimoda, Y., Nielsen, M. W., Jorgensen, F. G., Grossmann, C., Sandal, N., Sorensen, K., Thirup, S., Madsen, L. H., Tabata, S., Sato, S., Stougaard, J., and Radutoiu, S. 2010. Evolution and regulation of the *Lotus japonicus* LysM receptor gene family. Mol. Plant-Microbe Interact. 23:510-521.

Lopez-Lara, I. M., van den Berg, J. D., Thomas-Oates, J. E., Glushka, J., Lugtenberg, B. J., and Spaink, H. P. 1995. Structural identification of the lipo-chitin oligosaccharide nodulation signals of *Rhizobium loti*. Mol. Microbiol. 15:627-638.

Madsen, E. B., Madsen, L. H., Radutoiu, S., Olbryt, M., Rakwalska, M., Szczyglowski, K., Sato, S., Kaneko, T., Tabata, S., Sandal, N., and Stougaard, J. 2003. A receptor kinase gene of the LysM type is involved in legume perception of rhizobial signals. Nature 425:637-640.

Madsen, L. H., Tirichine, L., Jurkiewicz, A., Sullivan, J. T., Heckmann, A. B., Bek, A. S., Ronson, C. W., James, E. K., and Stougaard, J. 2010. The molecular network governing nodule organogenesis and infection in the model legume *Lotus japonicus*. Nat. Commun. 1, 10 doi:10.1038/ncomms1009. Published online.

Madsen, E. B., Antolin-Llovera, M., Grossmann, C., Ye, J., Vieweg, S., Broghammer, A., Krusell, L., Radutoiu, S., Jensen, O. N., Stougaard, J., and Parniske, M. 2011. Autophosphorylation is essential for in vivo function of the *Lotus japonicus* Nod Factor Receptor 1 and receptor mediated signalling in cooperation with Nod Factor Receptor 5. Plant J. 65:404-417.

Maillet, F., Poinsot, V., André, O., Puech-Pages, V., Haouy, A., Gueunier, M., Cromer, L., Giraudet, D., Formey, D., Niebel, A., Martinez, E. A., Driguez, H., Becard, G., and Dénarié, J. 2011. Fungal lipochitooligosaccharide symbiotic signals in arbuscular mycorrhiza. Nature 469:58-63.

Markmann, K., Giczey, G., and Parniske, M. 2008. Functional adaptation of a plant receptor-kinase paved the way for the evolution of intracellular root symbioses with bacteria. PLoS. Biol. 6:497-506.

Markmann, K., and Parniske, M. 2009. Evolution of root endosymbiosis with bacteria: How novel are nodules? Trends Plant Sci. 14:77-86.

Martinez-Romero, E. 2009. Coevolution in Rhizobium-legume symbiosis? DNA and Cell Biol. 28:361-370.

Masson-Boivin, C., Giraud, E., Perret, X., and Batut, J. 2009. Establishing nitrogen-fixing symbiosis with legumes: How many rhizobium recipes? Trends Microbiol. 17:458-466.

Mathesius, U. 2008. Auxin: At the root of nodule development? Functional Plant Biol. 35:651-668.

Mbengue, M., Camut, S., de Carvalho-Niebel, F., Deslandes, L., Froidure, S., Klaus-Heisen, D., Moreau, S., Rivas, S., Timmers, T., Herve, C., Cullimore, J., and Lefebvre, B. 2010. The *Medicago truncatula* E3 ubiquitin ligase PUB1 interacts with the LYK3 symbiotic receptor and negatively regulates infection and nodulation. Plant Cell 22:3474-3488.

Miche, L., Moulin, L., Chaintreuil, C., Contreras-Jimenez, J. L., Munive-Hernandez, J. A., Del Carmen Villegas-Hernandez, M., Crozier, F., and Bena, G. 2010. Diversity analyses of *Aeschynomene* symbionts in tropical Africa and Central America reveal that nod-independent stem nodulation is not restricted to photosynthetic bradyrhizobia. Environ. Microbiol.12:2152-2164.

Miya, A., Albert, P., Shinya, T., Desaki, Y., Ichimura, K., Shirasu, K., Narusaka, Y., Kawakami, N., Kaku, H., and Shibuya, N. 2007. CERK1, a LysM receptor kinase, is essential for chitin elicitor signaling in Arabidopsis. Proc. Natl. Acad. Sci. U.S.A. 104:19613-19618.

Morando, M. A., Nurisso, A., Grenouillat, N., Vauzeilles, B., Beau, J. M., Cañada, F. J., Jiménez-Barbero, J., and Imberty, A. 2011. NMR and molecular modelling reveal key structural features of synthetic nodulation factors. Glycobiol. 21:824-833.

Mukherjee, A., and Ané, J. M. 2011. Germinating spore exudates from arbuscular mycorrhizal fungi: Molecular and developmental responses in plants and their regulation by ethylene. Mol. Plant-Microbe Interact. 24:260-270.

Nakagawa, T., Kaku, H., Shimoda, Y., Sugiyama, A., Shimamura, M., Takanashi, K., Yazaki, K., Aoki, T., Shibuya, N., and Kouchi, H. 2011. From defense to symbiosis: Limited alterations in the kinase domain of LysM receptor-like kinases are crucial for evolution of legume-Rhizobium symbiosis. Plant J. 65:169-180.

Normand, P., Queiroux, C., Tisa, L. S., Benson, D. R., Rouy, Z., Cruveiller, S., and Medigue, C. 2007. Exploring the genomes of Frankia. Physiologia Plantarum 130:331-343.

Ohnuma, T., Onaga, S., Murata, K., Taira, T., and Katoh, E. 2008. LysM domains from *Pteris ryukyuensis* chitinase-A: A stability study and characterization of the chitin-binding site. J. Biol. Chem. 283:5178-5187.

Ohsten Rasmussen, M., Hogg, B., Bono, J. J., Samain, E., and Driguez, H. 2004. New access to lipo-chitooligosaccharide nodulation factors. Org. Biomol. Chem. 2:1908-1910.

Oka-Kira, E., and Kawaguchi, M. 2006. Long-distance signaling to control root nodule number. Curr. Opin. Plant Biol. 9:496-502.

Olah, B., Briere, C., Bécard, G., Dénarié, J., and Gough, C. 2005. Nod fac-

tors and a diffusible factor from arbuscular mycorrhizal fungi stimulate lateral root formation in *Medicago truncatula* via the DMI1/DMI2 signalling pathway. Plant J. 44:195-207.
Oldroyd, G. E., Mitra, R. M., Wais, R. J., and Long, S. R. 2001. Evidence for structurally specific negative feedback in the Nod factor signal transduction pathway. Plant J. 28:191-199.
Oldroyd, G. E. D., and Downie, J. A. 2006. Nuclear calcium changes at the core of symbiosis signalling. Curr. Opin. Plant Biol. 9:351-357.
Oldroyd, G. E., and Downie, J. A. 2008. Coordinating nodule morphogenesis with rhizobial infection in legumes. Annu. Rev. Plant Biol. 59:519-546.
Olsson, P. A., and Johansen, A. 2000. Lipid and fatty acid composition of hyphae and spores of arbuscular mycorrhizal fungi at different growth stages. Mycol. Res. 104:429-434.
Op den Camp, R., Streng, A., De Mita, S., Cao, Q., Polone, E., Liu, W., Ammiraju, J. S., Kudrna, D., Wing, R., Untergasser, A., Bisseling, T., and Geurts, R. 2010. LysM-type mycorrhizal receptor recruited for Rhizobium symbiosis in nonlegume *Parasponia*. Science 331:909-912.
Parniske, M. 2008. Arbuscular mycorrhiza: The mother of plant root endosymbioses. Nat. Rev. Microbiol. 6:763-775.
Petutschnig, E. K., Jones, A. M. E., Serazetdinova, L., Lipka, U., and Lipka, V. 2010. The Lysin Motif Receptor-like Kinase (LysM-RLK) CERK1 is a major chitin-binding protein in *Arabidopsis thaliana* and subject to chitin-induced phosphorylation. J. Biol. Chem. 285:28902-28911.
Radutoiu, S., Madsen, L. H., Madsen, E. B., Felle, H. H., Umehara, Y., Gronlund, M., Sato, S., Nakamura, Y., Tabata, S., Sandal, N., and Stougaard, J. 2003. Plant recognition of symbiotic bacteria requires two LysM receptor-like kinases. Nature 425:585-592.
Radutoiu, S., Madsen, L. H., Madsen, E. B., Jurkiewicz, A., Fukai, E., Quistgaard, E. M., Albrektsen, A. S., James, E. K., Thirup, S., and Stougaard, J. 2007. LysM domains mediate lipochitin-oligosaccharide recognition and Nfr genes extend the symbiotic host range. EMBO (Eur. Mol. Biol. Organ.) J. 26:3923-3935.
Redecker, D., Kodner, R., and Graham, L. E. 2000. Glomalean fungi from the Ordovician. Science 289:1920-1921.
Remy, W., Taylor, T. N., Hass, H., and Kerp, H. 1994. 4-hundred-million-year-old vesicular-arbuscular mycorrhizae. Proc. Natl. Acad. Sci. U.S.A. 91:11841-11843.
Shaw, S. L., and Long, S. R. 2003. Nod factor elicits two separable calcium responses in *Medicago truncatula* root hair cells. Plant Physiol. 131:976-984.
Shibuya, N., and Minami, E. 2001. Oligosaccharide signalling for defence responses in plant. Physiol. Mol. Plant Pathol. 59:223-233.
Shimizu, T., Nakano, T., Takamizawa, D., Desaki, Y., Ishii-Minami, N., Nishizawa, Y., Minami, E., Okada, K., Yamane, H., Kaku, H., and Shibuya, N. 2010. Two LysM receptor molecules, CEBiP and OsCERK1, cooperatively regulate chitin elicitor signaling in rice. Plant J. 64:204-214.
Shiu, S. H., Karlowski, W. M., Pan, R. S., Tzeng, Y. H., Mayer, K. F. X., and Li, W. H. 2004. Comparative analysis of the receptor-like kinase family in *Arabidopsis* and rice. Plant Cell 16:1220-1234.
Sieberer, B. J., Chabaud, M., Timmers, A. C., Monin, A., Fournier, J., and Barker, D. G. 2009. A nuclear-targeted cameleon demonstrates intranuclear Ca2+ spiking in *Medicago truncatula* root hairs in response to Rhizobial nodulation factors. Plant Physiol. 151:1197-1206.
Smit, P., Raedts, J., Portyanko, V., Debellé, F., Gough, C., Bisseling, T., and Geurts, R. 2005. NSP1 of the GRAS protein family is essential for rhizobial Nod factor-induced transcription. Science 308:1789-1791.
Smit, P., Limpens, E., Geurts, R., Fedorova, E., Dolgikh, E., Gough, C., and Bisseling, T. 2007. *Medicago* LYK3, an entry receptor in Rhizobial nodulation factor signaling. Plant Physiol. 145:183-191.
Sprent, J. I. 2007. Evolving ideas of legume evolution and diversity: A taxonomic perspective on the occurrence of nodulation. New Phytol. 174:11-25.
Staehelin, C., Charon, C., Boller, T., Crespi, M., and Kondorosi, A. 2001. *Medicago truncatula* plants overexpressing the early nodulin gene *enod40* exhibit accelerated mycorrhizal colonization and enhanced formation of arbuscules. Proc. Natl. Acad. Sci. U.S.A. 98:15366-15371.
Tirichine, L., Imaizumi-Anraku, H., Yoshida, S., Murakami, Y., Madsen, L. H., Miwa, H., Nakagawa, T., Sandal, N., Albrektsen, A. S., Kawaguchi, M., Downie, A., Sato, S., Tabata, S., Kouchi, H., Parniske, M., Kawasaki, S., and Stougaard, J. 2006. Deregulation of a Ca2+/calmodulin-dependent kinase leads to spontaneous nodule development. Nature 441:1153-1156.
Truchet, G., Roche, P., Lerouge, P., Vasse, J., Camut, S., de Billy, F., Promé, J.-C., and Dénarié, J. 1991. Sulphated lipo-oligosaccharide signals of *Rhizobium meliloti* elicit root nodule organogenesis in alfalfa. Nature 351:670-673.
Vasse, J., de Billy, F., and Truchet, G. 1993. Abortion of infection during the *Rhizobium meliloti*- alfalfa symbiotic interaction is accompanied by a hypersensitive reaction. Plant J. 4:555-566.
Walker, S. A., Viprey, V., and Downie, J. A. 2000. Dissection of nodulation signaling using pea mutants defective for calcium spiking induced by nod factors and chitin oligomers. Proc. Natl. Acad. Sci. U.S.A. 97:13413-13418.
Wan, J. R., Zhang, X. C., Neece, D., Ramonell, K. M., Clough, S., Kim, S. Y., Stacey, M. G., and Stacey, G. 2008. A LysM receptor-like kinase plays a critical role in chitin signaling and fungal resistance in *Arabidopsis*. Plant Cell 20:471-481.
Wang, B., Yeun, L. H., Xue, J. Y., Liu, Y., Ané, J. M., and Qiu, Y. L. 2010. Presence of three mycorrhizal genes in the common ancestor of land plants suggests a key role of mycorrhizas in the colonization of land by plants. New Phytol. 186:514-525.
Weidmann, S., Sanchez, L., Descombin, J., Chatagnier, O., Gianinazzi, S., and Gianinazzi-Pearson, V. 2004. Fungal elicitation of signal transduction-related plant genes precedes mycorrhiza establishment and requires the *dmi3* gene in *Medicago truncatula*. Mol. Plant-Microbe Interact. 17:1385-1393.
Xie, Z. P., Staehelin, C., Vierheilig, H., Wiemken, A., Jabbouri, S., Broughton, W. J., Vogellange, R., and Boller, T. 1995. Rhizobial nodulation factors stimulate mycorrhizal colonization of nodulating and nonnodulating soybeans. Plant Physiol. 108:1519-1525.
Xie, Z. P., Muller, J., Wiemken, A., Broughton, W. J., and Boller, T. 1998. Nod factors and tri-iodobenzoic acid stimulate mycorrhizal colonization and affect carbohydrate partitioning in mycorrhizal roots of *Lablab purpureus*. New Phytol. 139:361-366.
Xie, X., Yoneyama, K., and Yoneyama, K. 2010. The strigolactone story. Annu. Rev. Phytopathol. 48:93-117.
Yano, K., Shibata, S., Chen, W. L., Sato, S., Kaneko, T., Jurkiewicz, A., Sandal, N., Banba, M., Imaizumi-Anraku, H., Kojima, T., Ohtomo, R., Szczyglowski, K., Stougaard, J., Tabata, S., Hayashi, M., Kouchi, H., and Umehara, Y. 2009. CERBERUS, a novel U-box protein containing WD-40 repeats, is required for formation of the infection thread and nodule development in the legume-Rhizobium symbiosis. Plant J. 60:168-180.
Yokota, K., Fukai, E., Madsen, L. H., Jurkiewicz, A., Rueda, P., Radutoiu, S., Held, M., Hossain, M. S., Szczyglowski, K., Morieri, G., Oldroyd, G. E. D., Downie, J. A., Nielsen, M. W., Rusek, A. M., Sato, S., Tabata, S., James, E. K., Oyaizu, H., Sandal, N., and Stougaard, J. 2009. Rearrangement of actin cytoskeleton mediates invasion of *Lotus japonicus* roots by *Mesorhizobium loti*. Plant Cell 21:267-284.
Yokota, K., Soyano, T., Kouchi, H., and Hayashi, M. 2010. Function of GRAS proteins in root nodule symbiosis is retained in homologs of a non-legume, rice. Plant Cell Physiol. 51:1436-1442.
Zhang, B., Ramonell, K., Somerville, S., and Stacey, G. 2002. Characterization of early, chitin-induced gene expression in *Arabidopsis*. Mol. Plant-Microbe Interact. 15:963-970.
Zhang, X. C., Wu, X., Findley, S., Wan, J., Libault, M., Nguyen, H. T., Cannon, S. B., and Stacey, G. 2007. Molecular evolution of lysin motif-type receptor-like kinases in plants. Plant Physiol. 144:623-636.
Zhang, J., Subramanian, S., Stacey, G., and Yu, O. 2009. Flavones and flavonols play distinct critical roles during nodulation of *Medicago truncatula* by *Sinorhizobium meliloti*. Plant J. 57:171-183.
Zhang, X. C., Cannon, S. B., and Stacey, G. 2009. Evolutionary genomics of LysM genes in land plants. BMC Evol. Biol. 9:183. doi:10.1186/1471-2148-9-183. Published online.
Zhu, H. Y., Riely, B. K., Burns, N. J., and Ané, J. M. 2006. Tracing nonlegume orthologs of legume genes required for nodulation and arbuscular mycorrhizal symbioses. Genetics 172:2491-2499.
Zhukov, V., Radutoiu, S., Madsen, L. H., Rychagova, T., Ovchinnikova, E., Borisov, A., Tikhonovich, I., and Stougaard, J. 2008. The pea Sym37 receptor kinase gene controls infection-thread initiation and nodule development. Mol. Plant-Microbe Interact. 21:1600-1608.